

MultiScreen® Solubility Filter Plate

Performance and correlation of a 96-well high throughput screening method to determine aqueous drug solubility

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Abstract

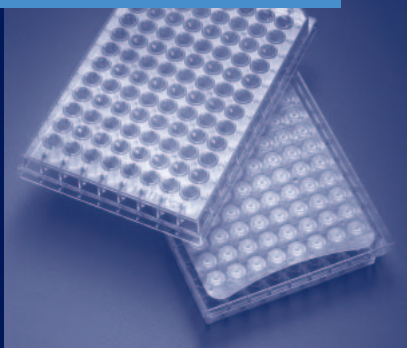
A high throughput screening method to classify the solubility of compounds using a new, 96-well filter plate (MultiScreen Solubility filter plate, Millipore, Danvers, MA) was evaluated to assess its correlation with standard (shake-flask) methodology.¹ The aqueous solubility of ten different commercially available compounds was measured multiple times in different MultiScreen Solubility filter plates. The performance of the assay and the correlation with shake-flask values are reported.

Background

Determining compound solubility in water has become an essential early measurement in the drug discovery process.^{2,3} Poor water-solubility can cause problems in many different *in vitro* testing techniques leading to unreliable results and/or reproducibility problems. Consequently, candidate compounds can fail early on in their development due to unfavorable physicochemical profiles. An even larger problem results when insoluble precipitates cause false positives in bioassays, potentially wasting valuable resources. Such issues can add significant cost and time to drug development activities.

The standard way to determine the solubility of a compound is to use the shake-flask solubility method.¹ This method is inherently low-throughput, labor intensive, and necessitates the addition of drug in powder form—a requirement which can be incompatible with how compounds are generally maintained (e.g., in DMSO^{3,5}). The shake-flask method involves adding an excess quantity of solid material to a volume of buffer at a fixed pH. This saturated solution is agitated (shake-flask) until equilibrium is reached, generally 24 to 48 hours. Following separation by filtration or centrifugation, the compound in solution is analyzed and quantified by UV/Vis spectroscopy or HPLC.

application note



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The MultiScreen Solubility filter plate has been designed and optimized for the determination of aqueous solubility in a high-throughput and automation-compatible workflow. The plate has been developed with the following attributes:

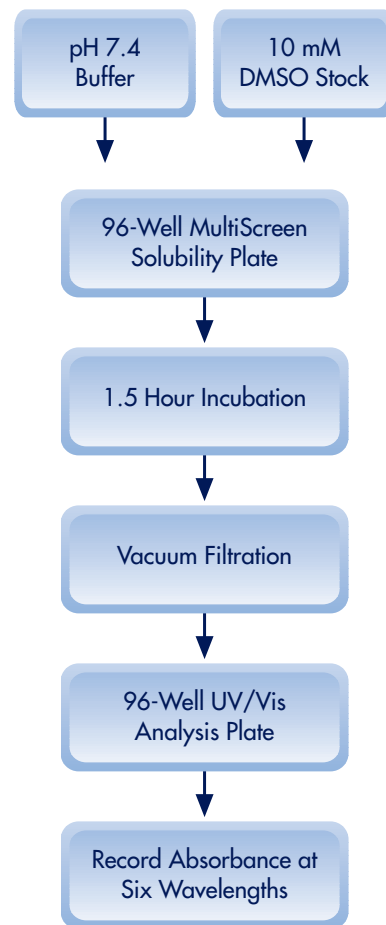
- 96-well format allows for solubility analysis of multiple drugs in a single plate
- Plate design compatible with all standard laboratory robotics and analytical equipment
- Low sample volume: 10 μL at 10 mM
- Direct quantitation of compound in solution
- Functional over a wide pH and excipient range
- High drug recovery provides reliable determination of soluble compound concentration
- Good particle retention removes insoluble compound
- Compatibility with aqueous organic solutions (e.g., $\leq 5\%$ DMSO in pH 3 – 12 buffers)
- Reproducible and repeatable results

Introduction

The MultiScreen Solubility filter plate with modified, track-etched, polycarbonate, 0.4 μm membrane, is a single-use, 96-well product assembly that includes a filter plate and a cover. The device is intended for processing 5% DMSO aqueous solubility samples in the 200 μL volume range. The vacuum filtration design is compatible with standard, microtiter plate, vacuum manifolds. The plate is also designed to fit with a standard, 96-well, microtiter, receiver plate for use in filtrate collection. The MultiScreen Solubility filter plate has been developed and is QC tested for consistent filtration flow-time (using standard vacuum), low aqueous-DMSO extractable compounds, high sample recovery properties and its ability to incubate samples as required to perform the solubility assay. The low-binding membrane has been specifically chosen for high recovery of dissolved organic compounds in aqueous media.

The aqueous solubility screening assay (see Figure 1) allows the estimation of a drug or a compound's aqueous solubility by mixing, incubating and filtering a solution in the MultiScreen Solubility filter plate. Solutions are filtered into a 96-well, collection plate using vacuum filtration and then analyzed by UV/Vis spectroscopy at six wavelengths. The relative solubility, in the form of a screening ratio, is then calculated using the sum of the recorded values as compared to a standard. The calculated screening ratio provides a fast method for identifying compounds that are highly, moderately or marginally soluble in aqueous solutions. As the screening ratio approaches unity, the sample is approaching the upper limit of solubility, 500 μM , as measured by the assay. If the screening ratio has a value less than 1 but greater than 0.5, the solubility of the compound is between 100 μM and 500 μM . A screening ratio of less than 0.5 indicates that the compound's solubility is likely to be less than 100 μM .

Figure 1: Aqueous Solubility Screening Assay



Test solutions are first prepared by adding an aliquot of concentrated drug or compound (typically 10 μL of 10 mM drug in DMSO) to 190 μL of buffer at a defined pH to achieve a final concentration of 500 μM in 5% DMSO. The buffer-drug solutions are mixed in a covered, 96-well, Multiscreen Solubility filter plate for 1.5 hours at room temperature. The solutions are then vacuum filtered into a 96-well, polypropylene (pp), V-bottomed collection plate to remove any insoluble precipitates. Upon complete filtration, 160 μL /well are transferred from the collection plate to a 96-well, UV/Vis analysis plate and diluted with 40 μL /well of acetonitrile. Absorbance of the analysis plate is read at six wavelengths using a UV/Vis microplate spectrometer to determine the absorbance profile of the test compound.

Materials

For materials, see Millipore Protocol Note PC2445EN00 entitled, "Determination of aqueous compound solubility using a 96-well filter plate to remove precipitated solids prior to UV/Vis spectroscopic analysis."

Protocol

- Prepare Universal Aqueous Buffer solution, pH 7.4, or a substitute buffer (see Millipore Protocol Note PC2445EN00), filter with a 0.22 μm Stericup™ filter unit to remove any particulates, and store at 4° C for up to one month prior to use.
- Dispense 190 μL /well of pH 7.4 buffer at room temperature into a MultiScreen Solubility filter plate with a multi-channel pipettor.
- Dispense 10 μL /well of stock compound in duplicate or triplicate (normally at 10 mM in DMSO, from a 96-well polypropylene, V-bottomed plate), directly into the buffer in the Multiscreen Solubility filter plate with a multi-channel pipettor. (For plate layout, see Millipore Protocol Note PC2445EN00, *Section III. 96-Well Aqueous Solubility Protocols*, Figure 6.) The final concentration of test compound in each well must be 500 μM .
- Cover the Multiscreen Solubility filter plate with a lid and mix with gentle shaking (100 – 300 rpm) at room temperature for 1.5 hours.
- While the test compounds are shaking, prepare the standards buffer consisting of a solution of 80:20 buffer:acetonitrile (AcN) to ensure overall compound solubility.
- Dispense 192 μL /well of room temperature, pH 7.4, buffer:AcN solution into a UV analysis plate with a multi-channel pipettor.
- Dispense 8 μL /well of stock compound (from the same 96-well polypropylene, V-bottomed plate as in step c) directly into the buffer in the UV/Vis analysis plate with a multi-channel pipettor. (For plate

layout see Millipore Protocol Note PC2445EN00, *Section III. 96-Well Aqueous Solubility Protocols*, Figure 6.)

- Cover the standards plate with a lid and mix with gentle shaking (100 – 300 rpm) at room temperature for 10 minutes.
- After mixing, read the standards plate with a UV spectrometer plate reader at six wavelengths: 280, 300, 320, 340, 360, and 800 nm.
- After mixing the Multiscreen Solubility filter plate for 1.5 hours, vacuum filter the solutions into a clean polypropylene, 96-well, V-bottomed, collection plate on a vacuum manifold with grid at 10 – 12" Hg. Filtration by vacuum requires that there is liquid in all 96 wells of the Multiscreen Solubility filter plate.
- After filtration, transfer 160 μL /well of filtrate to a clean UV/Vis analysis plate and dilute with 40 μL /well of acetonitrile.
- Cover the filtrate plate with a lid, and then mix with gentle shaking (100 – 300 rpm) at room temperature for 10 minutes.
- After mixing, read the filtrate plate with a UV/Vis spectrometer plate reader at six wavelengths: 280, 300, 320, 340, 360, and 800 nm.

Data Collection and Analysis

Data were collected using a Molecular Devices SPECTRAmax® Plus microplate spectrometer. For HTS results, single point UV/Vis absorbance spectra for compounds in 4% DMSO were obtained. The ratio of filtrate vs. standard absorbance was calculated to quantify the aqueous solubility using the formula below.

Results

Correlation

The screening ratios obtained from the MultiScreen Solubility filter plate screening method, as well as values obtained from the MultiScreen Solubility filter plate quantitative (see Millipore Protocol Note PC2445EN00 for quantitative protocol) and the shake-flask methods, are presented in Table 1. All solubility results were determined at pH 7.4 for each of the ten commercially available drugs. Solubility concentrations were determined from a five point standard curve for the quantitative and the shake-flask methods. Shake-flask aqueous solubility values were determined under standard conditions without modifications.

The correlation between the solubility concentrations approximated using the MultiScreen Solubility filter plate screening method and the shake-flask method is illustrated in Figure 2. In general, the MultiScreen Solubility

Aqueous Solubility Calculation

$$\text{If: } \frac{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm}) \text{ Filtrate}}{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm}) \text{ Standard}} \approx 1.00$$

Then: Aqueous Solubility \geq 500 μM

$$\text{If: } \frac{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm}) \text{ Filtrate}}{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm}) \text{ Standard}} \leq 0.5$$

Then: Aqueous Solubility \leq 100 μM

$$\text{If: } \frac{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm}) \text{ Filtrate}}{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm}) \text{ Standard}} < 1.00 \text{ and } > 0.5$$

Then: 100 μM < Aqueous Solubility < 500 μM

filter plate screening method over-estimates shake-flask solubility data. This positive bias, which is somewhat desirable in a screening method, is at least partially attributed to the low concentration levels of DMSO in the filter plate assay. In determining the solubility of a potential drug, a somewhat elevated value as compared to that measured by traditional methods is preferred. A low solubility value may lead to premature elimination of that potential drug candidate.

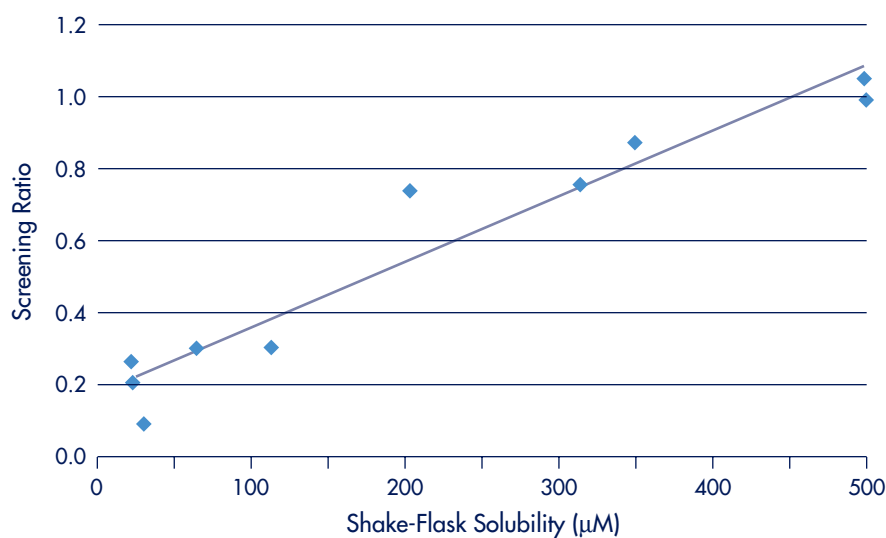
Conclusion

The MultiScreen Solubility filter plate screening method provides an automation compatible, high throughput means to estimate the aqueous solubility of hundreds of compounds per day. Using a single point calibration, the screening ratio is simply and quickly derived, and compound solubility is easily approximated. Multiple samples, each requiring approximately 200 nanomoles (~100 µg) per result, can be run in parallel. The method allows for the analysis of approximately 45 compounds (duplicate determinations) per plate with the capability of completing four or more plates in a standard 8-hour day. The assay is inherently compatible with the method by which most compound libraries are produced (e.g., as stock solutions in DMSO, etc.) and is easily integrated into existing chemical profiling and early ADME workflows.

Table 1: Comparison of solubility results: MultiScreen Solubility filter plates quantitative and screening methods vs. shake-flask solubility method.

Sample	Screening Ratio	Approximate Solubility (µM) from Screening Ratio	Solubility from Quantitative Method (µM)	Solubility from Shake-Flask Method (µM)
4,5-DPI	0.2	≤ 100	91	25
benzanthrone	0.26	≤ 100	63	23
β-estradiol	0.09	≤ 100	40	31
diethylstilbestrol	0.3	≤ 100	133	66
ketoconazole	0.3	≤ 100	123	114
griseofulvin	0.87	100 < [conc] < 500	393	350
phenazopyridine	0.73	100 < [conc] < 500	424	204
testosterone	0.75	100 < [conc] < 500	415	315
propranolol	1.04	≥ 500	498	500
verapamil	0.99	≥ 500	500	500

Figure 2: Correlation of screening ratio to shake-flask solubility.



References

1. ASTM: E 1148-02, *Standard Test Method for Measurements of Aqueous Solubility*, Book of Standards Volume 11.05.
2. Chait, A., *Discovery ADMET profiling: solubility technique*. Bioscience Tech., 2003. **05**: 33 – 34.
3. Bevan, C. D. and Lloyd, R. S., *A high-throughput screening method for the determination of aqueous drug solubility using laser nephelometry in microtiter plates*. Anal. Chem., 2000. **72**: 1781 – 1787.
4. Lipinski, C. A., Lombardo, F., Dominy B. W. and Feeney, P. J., *Experimental and computational approaches to estimate solubility and permeability in drug discovery and Development Setting*. Adv. Drug Delivery Rev., 2001. **46**: 3 – 26.
5. Ruell, J. and Avdeef, A., *A measured solution: Researchers are using different techniques to address drug solubility issues*. Mod. Drug Disc., 2003. p. 47 – 9.

Related Application and Protocol Notes

- PC2445EN00: Determination of aqueous compound solubility using a 96-well filter plate to remove precipitated solids prior to UV/Vis spectroscopic analysis
- AN1730EN00: Quantitative method to determine drug aqueous solubility: optimization and correlation to standard methods
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